

Association Between Maternal Serum Total Oxidant Status Total Antioxidant Status and Preterm Labor: A Prospective - Controlled Clinical Study

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OBJECTIVE: To measure the levels of individual antioxidant components of pregnant women with preterm labor to evaluate their total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI).

STUDY DESIGN: Prospectively-controlled 31 pregnant women with a diagnosis of preterm labor (group I) and 32 controls (group II) were evaluated for demographic data, general and obstetrical physical examination, obstetrical sonography, and routine laboratory tests. TAS, TOS and OSI levels were also measured.

RESULTS: There was no difference in terms of demographic data, ultrasound and routine laboratory parameters, total oxidant levels, total antioxidant capacity and oxidative stress index between the two groups ($p=0.621$, $p=0.706$ and $p=0.450$, respectively).

CONCLUSIONS: Improper balance between TAS and TOS may not be a major issue in the pathogenesis of preterm labor in which infection does not precede. 'Screening or prediction of preterm labor' needs new trials with large populations, particularly concerning environmental and dietary features of the population.

Key Words: Total antioxidant status, Preterm labor, Pregnancy

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Introduction

Preterm labor is a clinical condition due to adequate and frequent powerful contractions leading to progressive cervical dilation and effacement between 20th and 37th weeks of gestational age.¹ Preterm labor is the major problem in obstetrical practice due to both the high infant mortality and morbidity and to enormous economical health-care costs. Although many markers have been tested for the prediction of preterm labor, there is no widely accepted marker alone up to date. Inflammation is one of the major pathways in the pathogenesis of preterm labor.

In response to a chronic inflammatory insult (usually infections, but also possibly related to decidual hemorrhage) the fetal membranes and decidua produce cytokines (CRP,

TNF- α , IL-1, IL-6, IL-8, matrix metalloproteinase -8 and -9), which in turn initiate preterm labor.^{2,3} Prostaglandins stimulate uterine contractions; the metalloproteinases weaken the chorioamniotic membranes, and remodel and soften cervical collagen. Cytokines may aid in release of uterotonins (CRH, oxytocin).^{4,5}

Radical-scavenging antioxidants are consumed by the increased free radical activity associated with preterm labor, and the total antioxidant status (TAS) has been used to estimate free-radical activity.⁶ Various methods have been improved for the measurement of TAS. However, there is not yet an accepted 'gold standard' reference method,⁷ and decisions concerning standardization and the terms/units used for the measurement of TAS have not yet been made.^{8,9} TAS may help prevent preterm birth associated with inflammation and preterm labor. Inflammation enhances reactive oxygen species (ROS) and systemic inflammation produces severe oxidative stress, leading to tissue damage, resulting in preterm premature of the membranes (PPROM), and preterm labor.

In this study, we aimed to measure the levels of individual antioxidant components in venous blood samples from pregnant women with preterm labor to evaluate their TAS using a novel automated method designated as the Erel method.⁹ As a reciprocal measure, the total peroxide levels of the blood samples were measured. The percent ratio of the blood total oxi-

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dant status (TOS) to TAS level was regarded as the oxidative stress index (OSI).¹⁰

Material and Method

Study had been conducted at Gaziantep University, Faculty of Medicine, Department of Obstetrics and Gynecology after January, 2009; 31 pregnant diagnosed with preterm labor (group I), 32 gestational age-matched control pregnant (group II) were enrolled in the study. Study was approved by the Local Ethics Committee and informed consent was obtained from all subjects.

All pregnant subjects had singleton pregnancies without known pre-existing medical complications. Regular contraction frequency of six or more per hour, cervical dilation of >2 cm, effacement of >80 percent were considered as diagnostic criteria for preterm labor.

Pregnancies <20 and >37 weeks of gestation and pregnancies <37 weeks of gestation were excluded from the study groups I - II, and III - IV, respectively. Other exclusion criteria were presence of any fetal abnormality demonstrated by obstetric ultrasound, multiple pregnancies, intrauterine growth retardation, polyhydramnios, history of preterm birth, uterine abnormalities, smoking, premature rupture of membranes, systemic or local infections, increased C-reactive protein (CRP) levels, positive urinary culture, hypertensive disorders linked to pregnancy (preeclampsia, eclampsia) and diabetes (gestational or non-gestational).

Detailed history and demographic data (age, gravidity, parity, abortion) was obtained and gestational age was confirmed with early first trimester ultrasound for all subjects. Systemic and obstetrical examination was performed for all patients. Same researcher performed the examinations in order to prevent interobserver bias. Obstetrical ultrasound (Nemio 20, Toshiba, Tokyo, Japan) was also performed by the same researcher.

Cervical dilatation and effacement was recorded. All subjects were evaluated with external fetal tococardiography (Kranzbuhler, fetaCare® fetal monitor) for 10 minutes and fetal heart rates, uterine contraction frequencies and amplitudes were recorded. A Montevideo unit is the sum of the intensity of each contraction in a 10-minute period (in mmHG). Uterine contractions of the subjects in preterm labor group and term labor group were expressed as Montevideo Units.

Laboratory tests including complete blood count, urine analysis, urine culture, C-reactive protein (CRP), and assessments of serum levels of glucose, alanine aminotransferase (ALT), urea, calcium, electrolyte were all performed.

Venous blood samples of 5 mL were collected from of all subjects to silicon coated sterile vacuum tubes and processed

within 1 h with centrifugation at 1500 x g for 10 (min) at 4°C; samples were stored at -80°C until analysis (in less than 1 month) in centrifuge tubes.

TAS levels were measured using commercially available kits (Rel Assay). The novel automated method is based on the bleaching of characteristic color of a more stable ABTS (2,2'-azino-bis [3-ethylbenzothiazoline -6- sulfonic acid]) radical cation by antioxidants. The assay has excellent precision values, which are lower than 3%. The results were expressed as mmol Trolox (Rel Assay) equivalent/L.⁹ The Erel method can be performed by any fully automated biochemistry analyzer. Rel Assay trademark commercial kit is convenient for all autoanalyzers which determine fastness of measurement. In this study the Rel Assay analyzer, provided by Mega Tip (Gaziantep, Turkey), was used and the speed of the analyzer was 200 tests in 1 h. Rel Assay commercial kit work colorimetric method is based on 660 nm absorbance. The kit can also be used in manual spectrophotometer. As Rel Assay is the only fully automated kit, Erel method is faster and easier than existing kits to measure TAS.

TOS levels were measured using commercially available kits (Relassay, Turkey). In the new method, oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, which could be measured spectrophotometrically, was related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (mmol H₂O₂ equivalent/L).

The ratio of TOS to TAS was accepted as the OSI. For calculation, the resulting TAS unit was converted to mmol/L, and the OSI value was calculated according to the following formula: OSI (arbitrary unit)=TOS (mmol H₂O₂ equivalent/L) /TAS (mmol Trolox equivalent/L).⁹

Statistical analysis

Statistical analyses were performed with SPSS for Windows version 13.0 (SPSS Inc., Chicago, IL, USA). In preterm and normal pregnant women, multiple comparisons between groups were performed by oneway analysis of variance supplemented with Tukey's honestly significant difference (HSD) post hoc test. Demographic, clinical data of the subjects and TAS, TOS and OSI levels were evaluated with Student's t test. The association between TAS and TOS among groups was determined with Pearson's correlation test. Values of P < 0.05 were taken as significant. Data are presented as mean ± standard deviation (SD).

Results

There was no statistically difference was found in terms of demographic data, ultrasound and routine laboratory parameters between the groups.

The mean age of pregnant in preterm labor and gestational age-matched controls were, 28.42 ± 5.75 and 27.91 ± 6.61 , respectively ($p=0.744$). There was no statistically difference was found in terms of gravidity between the groups, 2.77 ± 1.38 and 2.97 ± 2.16 , respectively ($p=0.195$).

Mean gestational age of group I and II were 30.51 ± 4.89 and 28.40 ± 4.20 , respectively ($p=0.061$).

There was no statistically difference was found in terms of body max indices between the groups, 25.84 ± 4.45 and 25.48 ± 2.83 kg/m² ($p=0.706$). significant difference was found in terms of total oxidant levels, total antioxidant capacity and oxidative stress index between the two groups ($p=0.621$, $p=0.706$ and $p=0.450$, respectively) (Table I).

There was no cervical dilatation, effacement and uterine contraction in group II. In group I, mean cervical dilatation and effacement were 2.58 ± 0.63 cm and 40.5 ± 8.05 %.

There was no difference in terms of ultrasound parameters between preterm labor group and their gestational age-matched controls ($p>0.05$).

There was no significant difference between groups according to the laboratory values ($p>0.05$).

No significant difference was found in terms of total oxidant levels, total antioxidant capacity and oxidative stress index between the two groups ($p=0.621$, $p=0.706$ and $p=0.450$, respectively) (Table I). On the other hand there was a correlation between TAS and TOS values in both group I and II. In group I, there is an intermediate correlation between Total Oxidant Levels and Total Antioxidant Capacity ($r=0.456$, $p=0.010$). Similarly in group II, there is an intermediate correlation between Total Oxidant Levels and Total Antioxidant Capacity ($r=0.542$, $p=0.001$).

Discussion

An atom or a molecule capable of independent (usually short) existence containing one or more unpaired electrons is a free radical.¹¹ Free radicals generally belong to the broader category of ROS, such as singlet oxygen (O¹²), superoxide or hyperoxide anion radical (O₂⁻), perhydroxyl radical (HO₂), hydrogen peroxide (H₂O₂), hydroxyl radical (HO.), alcoxyl radical (RO), alcoxylradical (ROO), R-hydroperoxide (ROOH), ozone (O₃), nitric oxide (NO.), hypochlorous acid (HOCl), and peroxy nitrite anion (ONOO⁻). ROS are extremely reactive because of the presence of unpaired valence shell electrons.¹²

Increasing peripheral blood levels of superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, catalases, ascorbic acid, vitamin C, uric acid, protein thiols (albumin), bilirubin, transferrin, ceruloplasmin, cysteine, glutathione, and taurine, a- and g tocopherol, ubiquinol, b-carotene, and other carotenoids and oxycarotenoids and iron binding capacity levels during the course of pregnancy until term were reported reflecting the increase in antioxidant capacity.^{13,14}

Excessive ROS because of either increased ROS levels or reduced antioxidant defenses is termed oxidative stress. Pregnancy and miscarriage are conditions known to be associated with increased oxidative stress and increased levels of antioxidants seem to play a protective role in successfully ongoing pregnancies.¹⁵ Throughout gestation, circulating levels of lipid peroxidase increase markedly, as do other signs of oxidative stress such as leukocyte activation and increased plasma malondialdehyde.¹⁶ In normal physiology, ROS and antioxidant protection are in balance and ROS are needed as mediators and/or signal transducers in biochemical reactions. Excess production or poor control of oxidative stress may be involved in the etiology of obstetric complications like preeclampsia, PPRM etc.^{15,17} We therefore we aimed to measure the levels of individual antioxidant components in venous blood samples from pregnant women with preterm

Table 1: Total oxidant levels, total antioxidant capacity and oxidative stress index measurements

	Patient Group Mean ± SD Median (Min-Max)	Control Group Mean ± SD Median (Min-Max)	P
Total Antioxidant Status*	1.47 ± 0.16 $1.46 (1.22-1.76)$	1.45 ± 0.14 $1.45 (1.19-1.80)$	0.706
Total Oxidant Status**	4.89 ± 3.97 $4.30 (0.44-21.59)$	4.70 ± 2.63 $4.47 (0.22-11.87)$	0.621
Oxidative Stress Index (OSI)	0.33 ± 0.23 $0.26 (0.03-1.22)$	0.32 ± 0.16 $0.33 (0.02-0.68)$	0.450

*mmol Trolox Equiv./L, ** μ mol H₂O₂ Equiv./L

labor to evaluate their TAS and uncomplicated pregnancies at similar gestational weeks. Studies examining antioxidant substances separately are dominant in the literature.¹⁹⁻²⁰ The measurement of cumulative antioxidant status may be more significant than single components.²¹ Therefore, we measured total oxidative stress status.

One-third of preterm deliveries are attributed to PPRM, which can occur secondary to effect of inflammation and infection on fetal membranes, with one quarter of all preterm births are ascribed to microbial invasion.⁴ Oxidative stress may disrupt collagen and cause premature membrane rupture.²² ROS enhances the effects of inflammation and systemic inflammation produces severe oxidative stress, which in turn may lead to tissue damage, resulting in premature rupture of membrane and preterm labor.¹⁵

Antioxidants may aid in preventing preterm birth, which is associated with inflammation and preterm labor.^{15,23,24} Our exclusion criteria including premature rupture of membranes, systemic or local infections, increased C-reactive protein (CRP) levels and positive urinary culture probably eliminated most of these associations regarding infection/inflammation and preterm labor. Thus we could not designate any significant difference between the groups in terms of total oxidant levels, total antioxidant capacity and oxidative stress index.

The correlations between TAS and TOS in both groups reflect the balance between ROS and antioxidant protection. This issue raises concerns about the benefits of increase in nutrients or supplementary antioxidants such as vitamin C and E. Probably, improper balance between total oxidant levels, total antioxidant capacity may not be a major issue in the pathogenesis of preterm labor in which infection or premature rupture of the membranes do not precede.

'Screening or prediction of preterm labor' needs new trials with large populations, particularly concerning environmental and dietary features of the population.

Erken Doğum Eylemiyle Total Oksidan Seviye Total Antioksidan Kapasite ve Oksidatif Stres İndeksi Arasındaki İlişkinin Değerlendirilmesi: Prospektif - Kontrollü

AMAÇ: Erken doğum eylemi tanısı konulmuş gebeler ve gestasyonel haftası uyumlu kontrollerde total antioksidan kapasitelerini (TAK), total oksidan kapasitelerini (TOK) ve oksidatif stress indeksini (OSI) değerlendirmek.

GEREÇ VE YÖNTEM: Erken doğum eylemi tanısı konulan 31 gebe (grup I) ve gebelik haftaları ile uyumlu 32 gebelik kontrol grubu (grup II) çalışma kapsamında yer aldı. Tüm hastalarda demografik bilgiler, genel ve obstetrik muayeneler, obstetrik ultrasonografiler, ve rutin laboratuvar testleri uygulandı ve kay-

dedildi. Venöz kan örneğinden TAK, TOK ve OSI seviyeleri de değerlendirildi.

SONUÇLAR: Demografik bilgiler, ultrasonografi ve rutin laboratuvar parametreleri ($p>0,05$) ile TAK, TOK ve OSI değerleri yönünden farklılık gözlenmedi (sırasıyla $p=0,706$, $p=0,621$, $p=0,450$).

TARTIŞMA: TAK ve TOK arasındaki dengesizlik eğer altta yatan enfeksiyon veya erken membran rüptürü yoksa erken doğum eylemi patogenezinde majör bir rol üstlenmeyebilir. "Erken doğum eyleminin taramasında veya erken tanısında" özellikle çevresel faktörlerin ve beslenme özelliklerinin de dikkate alınıp çalışma popülasyonunun büyüklüğünün genişletildiği yeni çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Total antioksidan kapasite, Erken doğum eylemi, Gebelik

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